

**A STUDY OF CYTOCHROME C OXIDASE SUBUNIT 1 POLYMORPHISM
IN MALAY POPULATION**

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A STUDY OF CYTOCHROME COXIDASE SUBUNIT 1 POLYMORPHISM IN
MALAY POPULATION

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ABSTRACT

In the forensic science, genetic species identification based on DNA sequence differences and similarities will be used for crime survey. The mtDNA is much expanded for using as a marker of in species and individual identification. Cytochrome *c* oxidase subunit I is a part of mtDNA with 1548 base pair length. There are three different subunits of cytochrome *c* oxidase, COI, COII and COIII. Between the three mitochondrial genes which are coding the cytochrome *c* oxidase subunits, COI is the largest and the most conserved between them. This is the first study on the COI gene here in Malaysia. Cytochrome *c* oxidase subunit I (COI) was studied to check the COI gene polymorphism in Malay population and investigation the usefulness of this gene as forensic marker. For this aim, the blood samples were collected from 18 Malay people from different states. The PCR was done by using of extracted DNA from the blood and the COI gene was amplified with specific designated primers. Therefore, the desired region was revealed in the gel electrophoresis. The 13 nucleotide variations were observed within 1548 bp after sequencing of the samples and comparing with Anderson reference sequence.

ABSTRAK

Dalam bidang sains forensik, spesies genetic pengenalan berdasarkan perbezaan dan persamaan jujukan DNA akan digunakan bagi penyiasatan jenayah. Mt DNA banyak berkembang untuk digunakan sebagai penanda dalam spesies dan pengenalan individu. Cytochrome *c* oxidase subunit I adalah sebahagian daripada Mt DNA dengan 1548 pasangan asas panjang. Terdapat tiga jenis subunit berbeza: oxidase Cytochrome *c*, COI, gelung dan COIII. Antara tiga gen mitokondria yang mengekod subunit oxidase Cytochrome *c*, COI adalah yang terbesar dan yang paling dipelihara di antara mereka. Ini adalah kajian yang pertama pada gen COI di Malaysia. *c* oxidase subunit I Cytochrome (COI) telah dikaji untuk memeriksa polymorphism gen COI di kalangan penduduk Melayu dan penyiasatan kegunaan gen ini sebagai penanda forensik. Untuk tujuan ini, sampel darah telah dikumpul dari 18 orang Melayu dari negeri-negeri yang berbeza. PCR telah dilakukan dengan menggunakan DNA yang diekstrak daripada darah dan gen COI telah dikuatkan dengan primer tertentu yang ditetapkan. Oleh itu, rantau yang dikehendaki telah dikenalpasti dalam elektroforesis gel. 13 variasi nukleotida telah diperhatikan dengan saiz 1548 bp selepas urutan sampel dan dibandingkan dengan urutan rujukan Anderson.

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LIST OF ABBREVIATION

SYMBOLS	DESCRIPTION
bp	Base pair
<i>Cytb</i>	Cytochrome <i>b</i>
COI	Cytochrome <i>c</i> oxidase
DNA	Deoxyribonucleic acid
EDTA	Ethylendiaminetetraacetate
gr	Gram
HV1	hypervariable region 1
HV2	hypervariable region 2
kb	Kilobase
L	Litre
ml	Milliliter
°C	Degree centigrade
%	Percentage
rpm	Revolution per minute
STR	Short tandem repeats
SNP	single-nucleotide polymorphism
SSR	Simple Sequence Repeats
TAE	Tris-acetate-EDTA
µg	microgram
µl	microlitre
v/v	volume by volume
UTM	Universiti Teknologi Malaysia

CHAPTER 1

INTRODUCTION

1.1 Background of research

Mitochondria are organelles which is producing energy inside the cells. The energy will be produced through oxidative phosphorylation process by converting the fuels into ATP (Taanman; 1998). Mitochondria have a separate genome from nucleus genome which is called mitochondrial DNA (mtDNA). mtDNA is placed in the mitochondrial matrix. There are several copies of mtDNA in each mitochondrion organelle in mammalian cells (Michaels et al; 1982). MtDNA is small and has high copy number inside the cell and it will be easier to isolate the DNA from this organelle therefore, the first genome sequencing projects have been done on this molecule ([Http://megasun.bch.umontreal.ca](http://megasun.bch.umontreal.ca)). The sequencing projects of mtDNA molecule have been done in the huge number of species and also in human (Anderson et al; 1981).

MtDNA structure and genes organization are much conserved between mammals (Wolstenholme; 1992). Mammalian mtDNA is circular and closed, double-stranded

DNA molecule and the length of mtDNA is 16.6 kb. There are two strands on mtDNA molecule: heavy strand (H) and the light strand (L). The differentiation between them is based on G+C base composition. The difference G+C composition will result in different buoyant densities in different gradients of denaturing cesium chloride (Kasamatsu et al; 1974). The heavy strand (H) has the most coding information. Two rRNAs, 14 tRNAs and 12 polypeptide genes are on the heavy strand of mtDNA. There are eight tRNAs and a polypeptide gene was coded on the light strand (L).

Due to the low copy number of mtDNA in mammalian sperm cells and the extremely high copy number of mtDNA in mammalian oocyte cells, it is recognized that mtDNA in mammalian cells is transmitted through the female germ line (Hutchison et al; 1974, Giles et al; 1980, Gelfand et al; 1981)

In forensic science, genetic species identification based on DNA sequence differences and similarities will be used for crime investigation. The mtDNA is much expanded for using as a marker in species identification. The genes such as D-loop, cytochrome *b* and cytochrome *c* oxidase (COI) are more usual for this aim. In some cases, misidentification will happen due to the lack of primer specificity or incorrect reference sequence data from the available data (Dawnay et al; 2006). Some of the genes are very common for using in the forensic science and also species Identification such as cytochrome *b* (Verma and Singh; 2002, Branicki et al; 2003) and also the most variable or hyper-variable displacement loop region (which is known as D-loop) (Wu et al; 2005) of mtDNA. Some other genes also can be used as the forensic marker (Prieto et al; 2003, Prakash et al; 2000).

MtDNA is used for forensic identification due to the high copy number of this molecule in the cell. The high copy number of the mtDNA allowed the scientists to extract more concentration of DNA in comparison with nuclear DNA (Carracedo et al;

2000). In addition, mtDNA is usually used in phylogenetic studies due to Scientists believed that the substitution of nucleotide in the mtDNA has a constant rate through evolutionary time thus, they can estimate the divergence time by using of this feature (DeSalle et al; 1987).

Cytochrome *c* oxidase subunit 1 DNA size is around 1548 bp which has 70.2% total T content (Shaikevich and Zakharov., 1993). In addition, Cytochrome *c* oxidase protein (EC 1.9.3.1) is the last enzyme complex of respiratory electron transport chain which is located in the mitochondrial membrane (Valnotet *al.*, 2000). This enzyme is acting as a dioxygen activator in aerobic life by transferring the electron from the reduced cytochrome *c* to the oxygen (Valnotet *al.*, 2000). There are three different subunits of cytochrome *c* oxidase, COI, COII and COIII. Between the three mitochondrial genes which are coding the cytochrome *c* oxidase subunits, COI is the largest one and the most conserved between them (Beard *et al.*, 1993). Based on a survey about COI gene on some of the search engines such as GeneBank BLAST and BOLD, it can be concluded that the COI gene reliably identifies species where the reference sequence data is present (Dawnayet *al.*, 2006).

In the present study a survey about Cytochrome *c* oxidase subunit I (COI) has been done to evaluate COI as a marker for forensic identification on the Malay population in Malaysia. A recent study about the DNA barcoding suggested, the mitochondrial DNA gene, cytochrome *c* oxidase I (COI) can be used as a barcode for barcoding of the most animals (Hebert *et al.*, 2003).

Species identification were done by comparing and matching the sequence of evidence item to the reference sequence from the sequence similarities (Altschulet *al.*, 1997) and through the phylogenetic reconstruction (Baker and Palumbi., 1994). One of the most important limitations for species identification is the lack of valid reference

DNA sequence data with great sequence matches (Dawnay *et al.*, 2006). Cytochrome b and D-Loop sequences already submitted to the databases as part of phylogenetic and molecular ecological studies (e.g. Avise *et al.*, 2000) but they were generated and accepted without any standard protocols or quality control therefore, doubts over their suitability for forensic application were raised (Dawnay *et al.*, 2006).

The main purpose of the barcoding is COI reference sequences production which is from multiple specimens by using standard protocols. Through the developing of barcoding, in the future, the COI sequence application as a forensic marker can be predicted. An authentic genetic marker is a marker which is provide accurate results under different situations, otherwise, it cannot be validated marker (Dawnay *et al.*, 2006).

In this study, it has been tried to find out similarities and differences between the samples which can make COI gene sequence as a forensic marker for Malay populations.

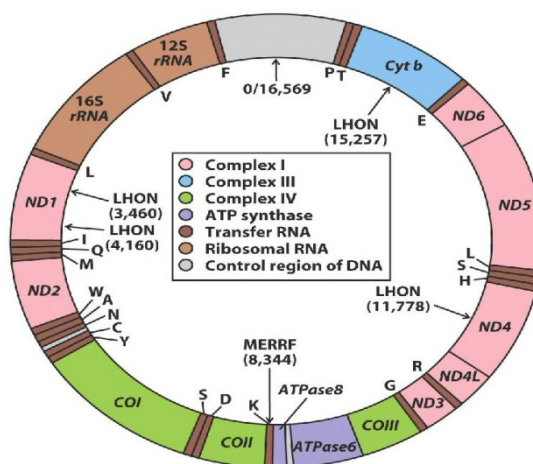


Figure1.1. Mitochondrial DNA ([Http://ashcrofthouse.org.uk](http://ashcrofthouse.org.uk))

1.2 Problem statement

Here in Malaysia, this is the first study about genetic polymorphism based on COI gene sequence but there were some previous studies about hypervariable regions (HV1 and HV2) polymorphisms in Malay population. This work is aimed to analyze the COI gene in the mtDNA in Malay people. Currently there are no established identification tools for Malay population at molecular level. Appearance and parents had been used to identify the Malay people and to differentiate between Malay, Chinese and Indian in Malaysian population and also between Malay to any other races up to date.

1.3 Objectives of the study

The objectives in this study were:

- I. To isolate human COI gene by using the specific primer.
- II. To compare the COI gene between different people within a population.
- III. To evaluate the suitability of COI as a marker for forensic aims.

1.4 Scope of study

The scope of this study is divided in to three main parts. The first part is blood sampling. The Malay people were selected as the donor and sample. 3ml of blood will be taken from each of donors. The second parts were laboratory works which is including DNA extraction, PCR and sequencing. The last part will be included bioinformatics analyzing and databases which is followed by alignment of sequences all together with the Anderson reference and finding out the polymorphisms places by using of JALVIEW software.

1.5 Significant of study

Based on the results of this study and the data which is prepared from this research, investigation about the COI can be continued. Other studies can investigate about these polymorphisms and find out if these polymorphisms can make any disease or not. These results will be used for forensic purposes in future and also they might be used in population genetic.

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